DETERMINATION OF THE DEVELOPMENTAL PATTERN OF ANGIOSPERMOUS LEAVES[1]

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In studies on the development of fruits in curcubits, Sinnott (1936) has shown the consequence of differential growth rates in two dimensions on the ultimate fruit form. Where the relative rate of growth of width and length remains constant throughout maturation, but width is increasing more, or less, rapidly than length, there will be major differences between the form of small and large fruits. The resulting difference in ultimate form may serve to mask what is a high degree of genetic uniformity. Under these circumstances, a single variable, the duration of growth, can produce marked differences in appearance. Comparable conditions certainly operate in respect to leaf development (Hammond 1941, Stephens 1944, Whaley and Whaley 1942). In the taxonomic analysis of species populations of angiospermous plants collected over a wide geographical range, and under different conditions of photoperiod and growing season, there is a wealth of potential information to be obtained from the developmental pattern of leaves. Unfortunately, most methods of studying leaf development require extensive periodic measurements, or anatomical sections. In order to reduce the time required for accumulation of such growth information, we have been experimenting in our laboratory with 3-amino triazole as a rapid method of showing the developmental pattern of leaves. Three-amino triazole (3-AT) interferes with the synthesis of chlorophyll in leaves (Pyfrom et al. 1957, Jukes 1963) but not with pre-existing chlorophyll molecules, and as a consequence, marks newly developed parts of the leaf through the absence of chlorophyll. From the interpretation of leaves caught at various stages of development at the time of application of 3-AT, the pattern of development — basipetal, acropetal or diffuse, — can readily be deter-

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mined. Moreover, by treating some leaves early in development, and others at later stages, it is possible to see the way in which leaf shape is changing, much more readily than through a comparison of unmarked leaves.

For many plants so far examined, an aqueous solution of 3-AT at concentrations of 0.13 grams per liter can be applied by spray or dropper directly to the apex of the plant without significantly altering the leaf size or form. The developmental pattern for a leaf of tomato, *Lycopersicon esculentum* Mill. variety Marglobe is shown in Fig. 1a. The pattern of development, which is basipetal both for the entire compound leaf, and for each lobe, is shown by the development of chlorophyll-free areas within the leaf. In a more detailed treatment of tomato, Breil (1963), Briel and Davis (1964) has shown that the pattern illustrated by 3-AT fits the developmental pattern as determined from anatomical studies and mitotic counts.

Daily measurement of growing leaves of plants of *Tiarella cordifolia* L., from New England and North Carolina, show the maximum length to maximum width ratio to remain almost constant at a value of 1 during the eight day period of principal leaf growth. During that period of growth, differences in leaf form, although minor, become accentuated. *Tiarella* plants from North Carolina show increased crenations on the leaf margin. Figure 1b shows a leaf in which 3-AT was applied early in development, and the effect on chlorophyll synthesis was wearing off in the final stages of leaf expansion. Consequently, chlorophyll appears only in the margin, which was the last part of the leaf to develop. Fig. 1c represents a drawing of a similar leaf, with the chlorophyll containing parts of the leaf in black. *Tiarella* plants from New England tend to stop expansion prior to this last phase of leaf growth, which occurs only in pockets at the margin of the leaf, and consequently show less pronounced crenations.

Equally as important as determining where leaf form does change during enlargement is establishing leaf characteristics which are constant throughout development. Figure 1d shows a photograph of a much dissected leaf of *Humulus*

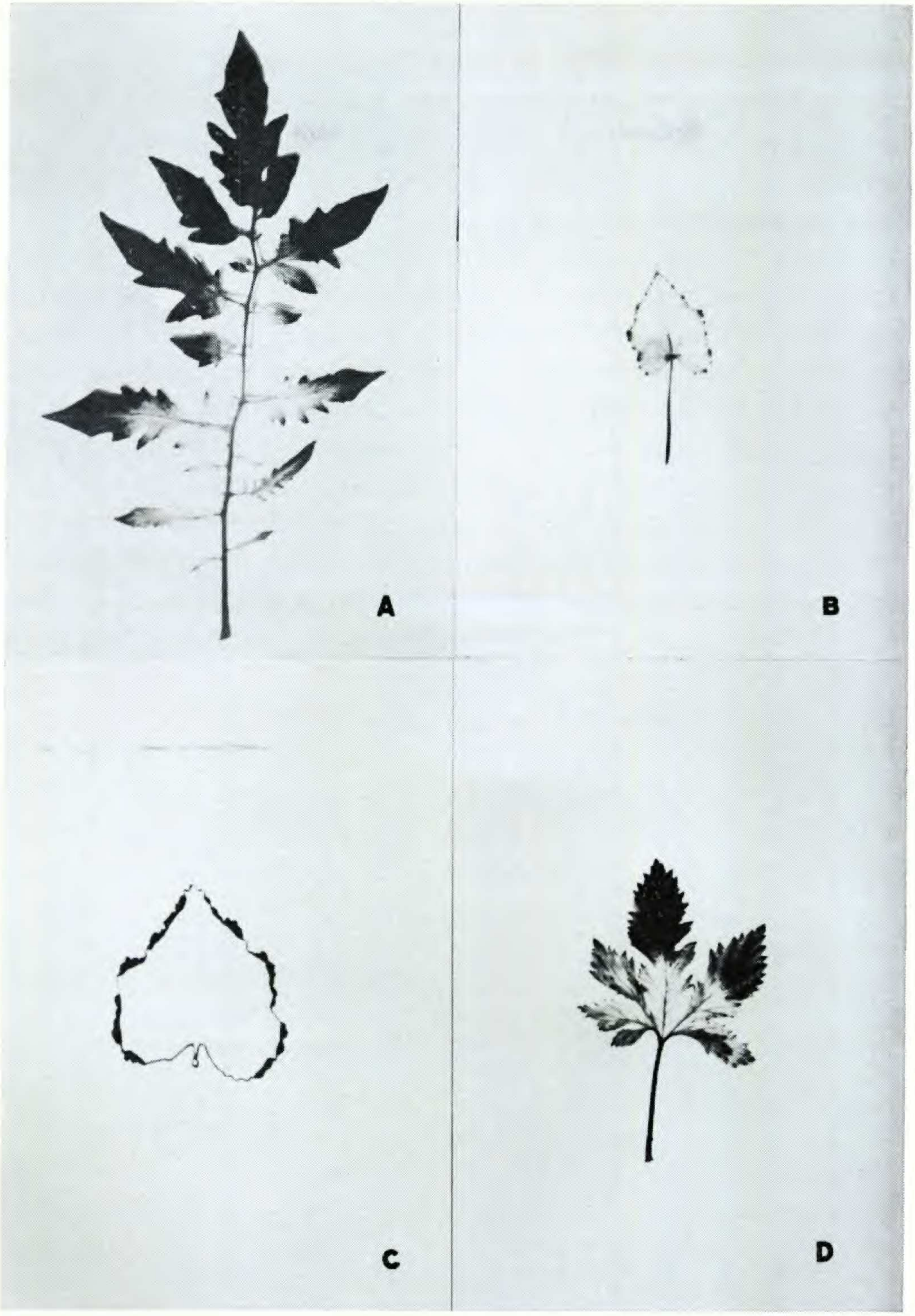

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Fig. 1. (a) Leaf of tomato treated with 3-AT; chlorophyll deficient areas at the base of lower lobes. (b) Treated leaf of *Tiarella*, chlorophyll containing areas at leaf margin. (c) Diagram of treated leaf of *Tiarella*, black areas corresponding to chlorophyll containing zones. (d) Treated leaf of *Humulus lupulus* from Rocky Mountains; central and right lobes showing normal growth; lateral lobes on left developed assymetrically and later than usual as indicated by absence of chlorophyll.

lupulus L. from the Rocky Mountains, treated with 3-AT at the mid-point in its development. The developmental sequence of the hop leaf is shown to be basipetal with the lobes maturing first, followed by the "palm" of the leaf. The form of the leaf has taxonomic significance for several hop varieties have been distinguished by the relative size of the lobes of the leaf. Daily measurements of the length of the lobes of the expanding leaf during a thirteen day period, from the time the leaves are first large enough to measure to completed expansion, show the ratio of the central lobe to the adjacent lateral lobe to be nearly constant within the range of 1.1 to 1.2 (Fig. 2), and its taxonomic use is independent of leaf age. This same information can be obtained more readily by measuring treated leaves at different stages

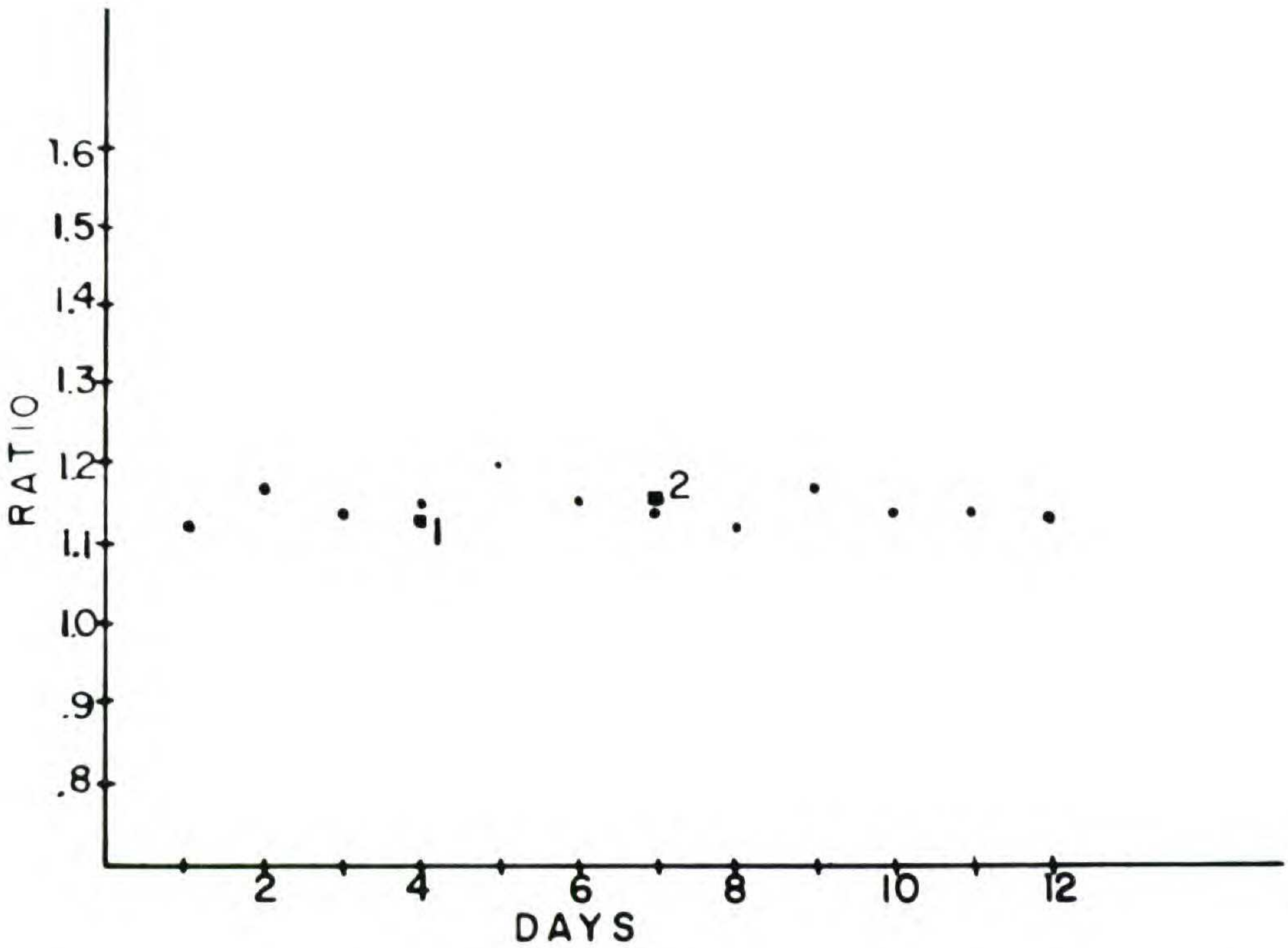

Fig. 2. Ratios of the length of the central lobe to the length of the adjacent lateral lobe plotted on the graph are for a single hop leaf, although ten leaves were measured in the experiment with comparable results, since the ratios are compared to a paired leaf treated with 3-AT. (1) ratio of green tissue at the time of treatment (prior to 3-AT effect) and (2) ratio of chlorophyll deficient tissue at time of leaf collection.

of expansion and determining on each leaf the ratio of the chlorophyll containing zones of the central and adjacent lobes, and making the same determination for the chlorophyll-deficient zones on these lobes. Where the expansion rate (as in hops) is uniform, calculations of the ratio of the tissues in each lobe formed before, or after, treatment for leaves at different stages of development give the same value. Fig. 2 also shows the ratios, determined by measuring green and colorless zones on a paired treated leaf at the time of treatment and at the time the leaf was collected 3 days later.

A comparison of the ratio during expansion of the central lobe to the second lateral lobe shows a marked decline as the leaf enlarges, as would be predicted from the treated leaves (cf. Fig. 1d) which show the basal part of the leaf to develop much later than the apical central portion. Thus with 3-AT it is possible to determine very rapidly those ratios for the leaf which may be independent of the duration or extent of leaf expansion.

It is not yet clear at what cellular stage 3-AT affects chlorophyll synthesis in developing leaves. Experiments are presently underway using tritiated thymidine to determine if only dividing cells, or both expanding and dividing cells are affected. Whatever the case, the use of an easily applied marker in leaf development, which permits a rapid recognition of newly expanding parts of the leaf blade, should make it possible to include more developmental information in taxonomic studies of plant populations.

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Errata for Rhodora Volume 66

p. 218 line 33 for *Fucus vesiculous* read *Fucus vesiculosus*
p. 223 in note at bottom, for *Pantoneura barii* read *Pantoneura bearii*
p. 234 line 20 for *P. plocamiodes* read *P. plocamioides*
p. 309 line 37 for *Basia* read *Bahia*
p. 312 line 9 for *Diosopyros* read *Diospyros*
p. 353 Fig. 7 is Plate 1306
p. 405 Fig. 1 is Plate 1307